Antiosteoporotic Effect of Some Herbal Extracts versus Alendronate on an Animal Model of Osteoporosis

Laila Sabry[1], Mai Abdul Sattar[2], Hanan Ali Amin[3*], and Essam Abdel Sattar[4]

Departments of Pharmacology[1, 2] and Anatomy[3], Faculty of medicine, King Abdul Aziz University
[2] Department of Pharmacology, Faculty of medicine, Ain Shams University.
[3] Department of Histology, Faculty of medicine, Cairo University.
[4] Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.
hananaliamin@hotmail.com

Abstract: The aim of the present study was to investigate the rationale for the use of two herbal extracts of *Sophora japonica* and *Calligonum comosum* whether alone or in combination versus alendronate in treatment of osteoporosis. Female albino rats were randomly arranged in eleven groups; 8 rats each. First group served as normal control while the second was sham operated. The remaining nine groups were ovariectomized (OVX); the first of which was not treated and served as a control for the other OVX and treated groups, while the second group received only DMSO (vehicle). The third group was treated with alendronate.Fourth, fifth; sixth and seventh groups were treated with Sophora and Calligonum at two dose levels respectively. In addition, eighth and ninth groups were treated with both plants at two dose levels. Alendronate and the extract of the herbs were administered orally daily for three months. Body weight was measured, biochemical effects were evaluated and histomorphometrical examination of the distal parts of the tibia. The results obtained revealed that OVX – treated rats exhibited a dose- dependent improvement in all measured parameters; body weight, biochemical markers, histomorphometic changes compared to non treated OVX-control group. Furthermore, combination of both plant extracts produced near results to that of alendronate improvement on biochemical markers and on cortical and trabecular bone thickness and this was a dose –dependent. Therefore, the present study clearly demonstrated that the extracts of Sophora Japonica and Calligonum Comosum have the potential for being used as alternative or supplement therapy for osteoporosis.
[Laila Sabry, Mai Abdul Sattar, Hanan Ali Amin, and Essam Abdel Sattar. **Antiosteoporotic Effect of Some Herbal Extracts versus Alendronate on an Animal Model of Osteoporosis**. *Life Sci J* 2013;10(4):177-187]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 23

Key wards: Osteoporosis, *Sophora japonica*, *Calligonum comosum* and Alendronate.

1. Introduction

Osteoporosis is a metabolic bone disease characterized by a reduction in bone mass and microarchitectural deterioration of bone tissue, resulting in skeletal fragility. Bone loss is progressive and is not associated with symptoms until a fracture occurs (Graham *et al.,* 2009).

Osteoporosis is a multifactorial disease whose susceptibility is determined by genetic and environmental factors, and hormonal status especially sex hormones, and certain disease and medications (Jakob *et al.,* 2008).

Bone remodeling is a dynamic process that occurs continuously throughout life. In healthy persons it is characterized by a balance between bone resorption and bone formation. At the cellular level, bone remodeling is regulated by osteoclast and osteoblast activity. During bone loss, there is an imbalance; osteoclast activity being more pronounced (Neumann, 2006).

Several over the counter drugs used in the treatment and prevention of osteoporosis increase bone density either by inhibiting resorption (calcium and vitamin D supplementation, bisphosphonates, strontium ranelate, hormone replacement therapy (HRT), raloxifen, calcitonin or increase formation(teriparatide) or by acting both ways. Each of these drugs has its limitation and is costly effective (Kapur *et al.,* 2008).

Bisphosphonates are a class of antiresorptive agents. These drugs are effective in reducing bone loss and fractures associated with postmenopausal osteoporosis. But the problem of bisphosphonates that it has complex administration requirements (fasting, ingestion with plain water only, post-dose fasting) and may cause upper gastrointestinal symptoms which may progress to esophageal cancer, intravenous bisphosphonates must be given by trained staff in a physician's office or hospitals (Amagase *et al.,* 2011).

Medicinal plants have been used widely used in clinical practice to treat bone disease for their safety and their potential antiosteoporotic effects. Herbal medication has different mechanisms of action as antiosteoporotic such as phytoestrogens, antioxidants, and anti-inflammatory and increase bone mineralization (Smietana *et al.,*, 2010 and Lacativa and Farias, 2010).

The herbs *Sophora japonica* and *Calligonum comosum* may have potential effectiveness in treating osteoporosis without producing the serious side effects of bisphosphonates. *Sophora japonica*; Family Fabaceae (Dried flowers and buds) is a medicinal herb used in China, Japan and Korea to treat hemorrhoids and hematemesis. *It reduces* cerebral infarction partly as a result of its anti-oxidative and anti-inflammatory activities. It contains five main flavonoids of rutin, quercetin, isorhamnetin, isorhamnetin, genistein and kaempferol (Tang *et al.,* 2002 and Chen and Hsieh, 2010). Moreover, *Sophora japonica* contains isoflavone glycoside such as sophoricosides (Kim *et al.,* 2003).

Calligonum comosum, an Egyptian desert plant belongs to family Polygonacaeae. In addition, it is shrub distributed throughout Saudi Arabia and growing in sandy desert.Ariel parts (stems and leaves) contain flavonoids kaempferol and quercetin (Liu *et al.,* 2001). The plant is woody based ascending shrub, glabrous much branched, erect up to 250 cm high, with minute leaves, and usually absent. *Calligonum comosum* is used in folk medicine to treat abdominal ailments; toothache and as hypoglycemic (El-Hawary *et al.,* 1990).The plant is used also as firewood which gives smokeless fires and for tanning. Previous studies on the aerial parts of *Calligonum comosum* were shown to possess anti-inflammatory, anti-ulcer, cytoprotective effects in rats (Liu *et al.,* 2001). Phytochemical investigation of *Calligonum Comosum* resulted in isolation and identification of catechin, dehydrodicatechin A, kaempferol-3-O-rhamnopyranoside, quercitrin, isoquercitrin, kaempferol-3-O-glucuronide, and mequilianin (Badria *et al.,* 2007).

Thus, the aim of the present study was to investigate the antiosteoporotic potential of these two herbal extracts either alone or in combination versus alendronate in an experimental model of osteoporosis. The results were assessed by estimating serum alkaline phosphatase, serum calcium, serum phosphorus and parathyroid hormone. Moreover, histological and morphometric studies were carried out.

2. Material and Methods

I- Material:

A. Animals: A total number of 88 adult female albino rats, weighing between 220-270 g at the beginning of the study were used. The animals were kept under normal laboratory conditions, and given free access of food and water.

B. Test drug: Alendronate sodium: The powder was dissolved in distilled water and given in a dose of 0.9mg/kg/day by an intragastric tube. This dose was extrapolated from the proposed human therapeutic dose according to Paget and Barnes (1964).

C. Plant material: *The aerial parts of Calligonum comosum* were collected from Egypt in May 2010. The plant materials were air-dried and subjected to grinding, then kept in dark air-tight closed containers until extraction step. In addition, the fruits of *Sophora japonica* were collected from the Experimental Station of Faculty of Pharmacy, Cairo University, in April 2010. The plant materials were air-dried and subjected to grinding, then kept in dark air-tight closed containers until they were extracted with methanol (3 × 2000 ml) using Ultraturrax T25 homogenizer. The solvents were distilled off under reduced pressure to give 152 g of yellowish brown extract, which then lyophilized and kept at 4 ºC till biological tests.

II- Methods:

Experimental design:

Female albino rats were randomly arranged in eleven groups; 8 rats each. First group serves as normal control for ovariectomized(OVX) groups while the second was sham operated(ovaries were only exposed to induce a stress similar to that obtained with bilateral ovariectomy). All other nine groups were OVX; the first of which were not treated and served as a control for the other OVX and treated groups. Moreover, the second group was treated with Dimethyl sulfoxide (DMSO). The third group was treated with alendronate at a dose of 0.9mg/kg/day. Fourth and fifth group was treated with *Sophora Japonica* at two dose levels (100 and 200 mg/kg/day respectively) while the sixth and seventh were treated with *Calligonum Comosum* at two dose levels (100 and 200 mg/kg/day respectively). In addition, eighth and ninth was treated with both plants at the two dose levels used. This was maintained for three months.

Body weight measurements for all animals groups were done once each week. At the end of the treatment the animals were *anesthetized by diethyl ether and* sacrificed. Blood was collected for serum analysis and tibiae were kept in neutral buffered formalin for histological and morphometric analysis.

Evaluation parameters:

Biochemical evaluation:

After three months of ovariectomy, serum samples were collected for the measurement of indices of bone turnover (serum calcium (mM/L), serum phosphorous (mM /L), serum alkaline phosphatase (unit (u)/L), and parathyroid hormone (picogram (Pg)/L) which were assessed by using Hitachi 902 system, Roche, Mannheim.

Histological evaluation:

The left tibia from each animal was dissected and cleaned from soft tissues. Tibiae were fixed in neutral buffered formalin and samples were processed and embedded in paraffin blocks. Three micrometer sections of the blocks for all the treatment groups were cut and stained with haematoxylin and eosin.

Quantitative Histomorphometric evaluation:

Bone histomorphometric study was performed by using image analysis system Leica Q 500 MCO analyzer. Five fields from each slide of five different animals from each group were assessed. The thickness of the outer compact bone at the diaphysis and cancellous bone trabeculae at the metaphysis of the distal end of the tibia were measured.

These parameters were compared with the control group, non treated OVX group and other groups. Body weight was also measured and compared between all groups. Furthermore, after three months of treatment, the curative effect of plant extracts versus alendronate in OVX induced osteoporotic female rats was evaluated by measuring the above mentioned parameters. The results were compared between control group, non treated OVX group and all treated groups.

3. Results

Body weight changes (gm) in various groups of ovx- rats:

Table (1) and Fig (1) demonstrates the changes in the body weight of experimental groups of female albino rats after three months of treatment.

The final recorded body weight for various groups was significantly decreased compared to Ovx control group while there was a non significant increase in body weight in comparison to pre-ovariectomized control after 3 months of ovariectomy.

As shown from table (1) that the body weight was significantly (p<0.05) increased for animals in the OVX-control group and OVX-DMSO group compared to control group. While a non significant (p>0.05) decrease in the body weight was observed in the sham operated control group compared to control group after six months of ovariectomy.

As demonstrated in table (1) that oral treatment for three months with alendronate (0.9mg/kg/day), *Sophora japonica* (100mg/kg/day and 200mg/kg/day), *Calligonum comosum* (200mg/kg/day), or combined oral treatment with both plants at two doses level (100 - 200mg/kg/day) resulted in a dose-dependent and significant (p<0.05) decrease in body weight with respect to OVX-control group. Moreover, there was non significant (p>0.05) increase compared to control group.

When applying ANOVA followed by Tukey's test for comparison between alendronate (0.9mg/kg/day) treated group and other treated groups there was a non significant (p>0.05) change in body weight of all treated groups.

Biochemical changes in various groups of ovx- rats:

Collective Table (2) demonstrated changes in biochemical parameters; serum phosphorus, serum calcium, serum alkaline phosphatase and serum parathyroid hormone in different Ovx- rats groups after three months of treatment.

Table (2) demonstrated increases in bone turnover in the non treated ovx -group after six months of ovariectomy compared to pre -ovx group as observed by significant (P <0.05) increase in serum alkaline phosphatase and the compensatory non significant (P >0.05) increases in the parathyroid hormone while a non significant (P >0.05) decrease in serum calcium and increase in serum phosphorous were found.

While oral treatment with alendronate (0.9mg/kg/day) for three months produced improvement in bone biochemical markers compared to non treated ovx -control group. Serum calcium, serum alkaline phosphatase levels were significantly (P <0.05) decreased while serum parathyroid hormone and was significantly (P <0.05) increased. Moreover, serum phosphorouse level was non significantly (P >0.05) increased.

Furthermore, *Sophora japonica* exhibited a dose-dependent antiosteoporotic activity in the rat model used. Where non significant (P >0.05) changes in all bone biochemical markers were observed in the two dose levels used comparing to non treated ovx -control group.

In the present work, oral treatment with *Calligoum comosum* for three months exhibited a dose-dependent improvement in all biochemical markers compared to non treated OVX-control group.

Moreover, in the present investigation, oral treatment with both plants at the two dose levels used (100mg/kg/day and 200mg/kg/day) for three months produced improvement than either of them alone in bone biochemical markers versus non treated ovx -control group.

When applying ANOVA test followed by Tukey's test for comparison between alendronate (0.9mg/kg/day) treated group and other treated groups it was only found significantly (P <0.05) increased in serum calcium and serum alkaline phosphatase in *Sophora japonica* (100 and 200 mg/kg/day) and *Calligonum comosum* (100mg/kg/day) treated groups. Moreover, it was found only significantly (P <0.05) decreased in serum phosphorous with both plants (100mg/kg/day). In addition, there was a non significant (P >0.05) increases in serum parathyroid in both plants treated group at two dose levels while a non significant (P >0.05) decrease with all other treated group.

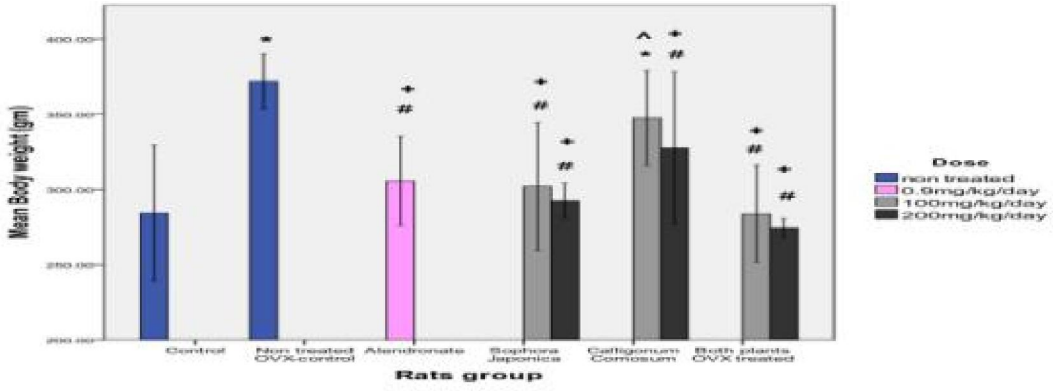

Figure (1): Body weight changes (gm) in treated groups [alendronate (0.9mg/kg/day), *Sophora japonica* (100 and 200mg/kg/day), *Calligonum comosum* (100 and 200mg/kg/day), and both plant extract (100 and 200mg/kg/day)]of female albino rats compared to control group and non treated OVX-control group (number of animal=8/group). Data expressed as means±SD., $^{+}$ non significant (P>0.05), * Significant(P < 0.05) versus pre -ovx group. $^{\wedge}$ non significant (P >0.05), $^{\#}$ Significant(P < 0.05) versus OVX-control group.

Table (1) Body weight changes (gm) in various experimental groups of ovx- rats

Group	Body weight changes (g) (mean ± SD)
Control	284.37 ± 45.23
OVX -control	371.87 ± 18.31*
Sham	253.75 ± 25.46^{+}
OVX + demso	338.75 ± 22.79*
OVX + alendronate	305.62 ± 29.45$^{\#+}$
OVX + sohpra 100mg/kg	301.87 ± 42.58$^{\#+}$
OVX + sohpra 200mg/kg	292.50 ± 11.64$^{\#+}$
OVX + calligonum 100mg/kg	347.50 ± 31.50$^{\wedge *}$
OVX + calligonum 200mg/kg	327.50 ± 50.63$^{\#+}$
OVX + both plants 100mg/kg	283.75 ± 32.37$^{\#+}$
OVX + both plants 200mg/kg	274.37 ± 6.23$^{\#+}$

$^{+}$ Non significant (P >0.05), * Significant(P < 0.05) versus corresponding control group.
$^{\wedge}$ Non significant (P >0.05), $^{\#}$ Significant(P < 0.05) versus non treated OVX-control group.

Table (2) Biochemical changes in various groups of ovx- rats:

	Calcium 2.20-2.62 mM/L	Phosphorous 0.8-1.60 mM/L	Alkaline phosphatase 50-136 u/L	Parathyroid hormone 45 - 163 Pg/L
Control (mean± SD)	2.56 ± 0.40	1.52 ± 0.08	100.5 ± 5.92	130.52±10.51
OVX control After three months of treatment (mean± SD)	2.52 ± 0.02^{+}	1.60 ± 0.13^{+}	143.75 ± 29.14*	142.90 ± 18.08^{+}
Sham (mean± SD)	2.55 ± 0.05^{+}	1.53 ± 0.05^{+}	98.75 ± 6.81^{+}	132.12 ± 12.98^{+}
OVX + Demso (mean± SD)	2.52 ± 0.06^{+}	1.58 ± 0.07^{+}	141.25 ± 20.17*	142.87 ± 17.66^{+}
OVX + alendronate (mean± SD)	2.41 ± 0.08$^{\#*}$	1.61 ± 0.04$^{\wedge *}$	90.00 ± 14.80$^{\#+}$	166.72 ± 9.92$^{\#*}$
OVX + Sohpra 100mg/kg (mean+ SD)	2.51 ± 0.04$^{\wedge +}$	1.58 ± 0.31$^{\wedge +}$	142.37 ± 25.09$^{\wedge *}$	154.37 ± 7.68$^{\wedge *}$
OVX + Sohpra 200mg/kg (mean± SD)	2.49 ± 0.03$^{\wedge *}$	1.69 ± 0.21$^{\wedge +}$	134.25 ± 12.08$^{\wedge *}$	150.37 ± 9.22$^{\wedge *}$
OVX + Calligonum 100mg/kg (mean± SD)	2.50 ± 0.02$^{\wedge *}$	1.60 ± 0.04$^{\wedge +}$	121.56 ± 13.02$^{\wedge *}$	150.62 ± 19.24$^{\wedge *}$
OVX + Calligonum 200mg/kg (mean± SD)	2.48 ± 0.04$^{\#*}$	1.62 ± 0.08$^{\wedge *}$	116.25 ± 13.02$^{\#*}$	154 ± 8.76$^{\wedge *}$
OVX + both plants 100mglkg (mean± SD)	2.43 ± 0.06$^{\#*}$	2.06 ± 0.23$^{\#*}$	99.75 ± 22.19$^{\#+}$	158.50 ± 18.23$^{\wedge *}$
OVX + both plants 200mglkg (mean± SD)	2.43 ± 0.03$^{\#*}$	1.69 ± 0.22^{++}	96.50 ± 9.95$^{\#+}$	161.50 ± 16.67$^{\wedge *}$

$^{+}$ non significant (P >0.05), * Significant(P < 0.05) versus control group. $^{\wedge}$ non significant (P >0.05), $^{\#}$ Significant(P < 0.05) versus non treated OVX-control group.

Morphometric results:

The mean cortical and trabecular bone thickness revealed a significant decrease (P <0.05) in the non treated OVX group (group III) compared to control group (group I) which indicated bone loss. Whereas, oral treatment with alendronate resulted in a significant (P <0.05) increase in cortical and trabecular bone thickness compared to non treated OVX group as shown in table (3). It also presented a significant (P <0.05) increase in groups IV- IX (receiving *Sophora japonica* or *Calligoum comosum*

at two dose levels compared to non treated OVX group.

Furthermore, a significant (P <0.05) increase in cortical and trabecular bone thickness were also observed in groups X-XI treated by combination of both plants at the two dose levels used compared to non treated OVX group.

Table (3) the mean outer cortical bone thickness and trabecular thickness in the different groups of ovx- rats:

Rats group	Cortical bone Thickness (Mean µm ± SD)	Trabecular bone Thickness (Mean µm ± SD)
Control group	258.4 ± 20.4$^{\#}$	93.1 ± 10.2$^{\#}$
Sham Operated	249.2 ± 19.5$^{\#}$	88.9 ± 13.1$^{\#}$
Non treated OVX	199.8 ± 25.4*	41.9 ± 11.5*
OVX + DMSO	209.1 ± 21.6*	45.8 ± 12.5*
Alendronate (0.9mg/kg/day)	250.6 ± 27.8$^{\#}$	87.6 ± 11.8$^{\#}$
***Sophora japonica* 100mg/kg/day**	220.1 ± 23.3$^{\#}$*	57.1 ± 10.8$^{\#}$*
***Sophora japonica* (200mg/kg/day)**	225.6 ± 18.5$^{\#}$*	63.5 ± 15.4$^{\#}$*
***Calligonum comosum* (100mg/kg/day)**	232.5 ± 29.8$^{\#}$	67.6 ± 11.3$^{\#}$*
***Calligonum comosum* (200mg/kg/day)**	237.8 ± 22.1$^{\#}$	69.9 ± 18.7$^{\#}$*
Both Plants (100mg/kg/day)	243.6 ± 27.8$^{\#}$	77.6 ± 11.8$^{\#}$
Both Plants (200mg/kg/day)	260.1 ± 25.1$^{\#}$	87.1 ± 15.1$^{\#}$

$^{+}$ non significant (P>0.05), * Significant(P < 0.05) versus control group. $^{\wedge}$ non significant (P>0.05), $^{\#}$ Significant(P < 0.05) versus non treated OVX-control group.

Histological results:

Control group and sham-operated group:

Hematoxylin and eosin-stained sections of the distal tibia diaphysis of control rats revealed that it was formed of an outer shell of cortical bone to which the periosteum was attached to its external surface and endosteum was attached to its internal surface. The periosteum was composed of a thick outer fibrous layer that was formed of dense collagenous fibers with fibroblasts in between the fibers and an inner osteogenic layer. The outer cortical bone appeared as a layer of compact bone containing blood vessels and osteocytes inside their lacunae. The cortical bone showed subperiosteal bone deposition appearing as a distinct basophilic cement line demarcating the border between newly added bone matrix and the older bone (Figure 1 A).

The endosteal surface of the cortical bone appeared smooth and was lined with osteoprogenitor cells, osteoblasts and osteoclasts residing in their Howship's lacunae (Figure 1 B).

The inner cancellous bone of the distal tibia metaphysis of control rats consisted of a network of bony trabeculae separated by interconnecting spaces containing bone marrow. The bone trabeculae consisted of irregular bone lamellae and osteocytes within their lacunae in between bone lamellae. Cement lines were observed as basophilic lines, and the matrix of some trabeculae showed more basophilic stainability (Figure 1C).

There was no apparent histological difference between control group and sham operated group.

Non treated-OVX group and OVX + DMSO group:

Examination of sections of the distal tibia diaphysis of rats of this group revealed no signs of subperiosteal new bone deposition showing indistinct basophilic cement lines in the cortical bone compared with controls. Resorption cavities were observed in the cortical bone and erosion cavities were detected at endosteal surfaces of cortical bone (Figure 1D, 1E).

The inner cancellous bone trabeculae lost their normal architecture and appeared as discontinuous bony ossicles separated by widened bone marrow spaces. Some bone trabeculae showed irregular eroded surface and others were observed as island of widely separated specules. Other trabeculae appeared thinned out compared with controls showing areas of decreased density showing faintly stained bone matrix (Figure 1F).

There was no apparent histological difference between non treated OVX-control and OVX + DMSO treated group.

OVX- alendronate (0.9mg/kg/day)- treated group*:*

Examination of the hematoxylin and eosin-stained (H&E) sections of the distal tibia diaphysis of this group revealed that the outer cortical bone was apparently similar to that of the control group, but differed from non treated OVX-control. The cortical bone contained osteocytes within their lacunae and many blood vessels. It showed smooth endosteal surface and subperiosteal bone deposition showing a distinct basophilic cement line (Figure 1G).

Moreover, the inner cancellous bone presented almost no apparent widening in the interconnecting bone marrow spaces. The cancellous bone trabeculae presented distinct deep basophilic cement lines and areas in the core of the trabeculae (Figure 1H).

OVX – Sophora japonica (200 mg/kg/day)-treated group:

H&E stained sections of the distal tibia diaphysis of rats of this group revealed the presence of resorption cavities in the cortical bone. The main difference from non treated OVX-control group was the presence of deep basophilic areas and cement lines around these cavities (Figure 1I).

Sections of distal metaphysis of tibia of rats of these groups showed improvement of cortical bone thickness (Figure 1I). The treatment with *Sophora japonica* 200 mg/kg/day was slightly potent than that with *Sophora japonica* 100mg/kg/day in protecting against osteoporosis in OVX rats.

The inner cancellous bone trabeculae are thinned out and separated by widened bone marrow spaces. Apparent increase in the thickness of the outer fibrous layer of the periosteum was also noted (Figure 1J).

On the other hand, very mild non significant changes versus non treated OVX-control group were observed (data not shown).

OVX - Calligonum comosum (200 mg/kg/day)-treated group:

The treatment with *Calligonum comosum* 200mg/kg/day was more potent than that with *Calligonum comosum* 100mg/kg/day in protecting against osteoporosis in OVX rats.

H&E stained sections of distal metaphysic of tibia of rats of these groups showed almost preservation of cortical bone thickness with the appearance of few osteoporotic cavities (Figure1K).

The cancellous bone trabeculae almost regained their normal architecture with less widening of the interconnecting bone marrow spaces as compared to non treated OVX-control. Distinct resorption areas in the core of the trabeculae were present (Figure 1L).

On the other hand, very mild non significant changes versus non treated OVX-control group were observed (data not shown).

OVX + both plants (200 mg/kg/day)-treated group:

The treatment with both plants 200 mg/kg/day was more potent than both plants 100mg/kg/day in protecting against osteoporosis in OVX- rats.

H&E stained sections of distal metaphysic of tibia of rats of these groups showed almost preservation of cortical bone thickness (Figure 1M).

The cancellous bone trabeculae almost regained their normal architecture with less widening of the interconnecting bone marrow spaces as compared to non treated OVX group. More distinct basophilic areas in the core of the trabeculae as compared to non treated OVX-control were present (Figure 1N).

4. Discussion

Bone fragility increases as age advances because of the appearance of abnormalities in bone remodeling. The negative balance between the relatively greater volume of bone resorbed by osteoclasts than that subsequently deposited by osteoblasts in the basic multicellular unit combined with a high rate of bone remodeling produce trabecular thinning, loss of trabeculae, cortical porosity and cortical thinning (Rizzoli, 2010). Alendronate is an antiresorptive agent which reduces the rate of remodeling by about 60% (Rizzoli, 2010). However, in experimental animals, several studies have demonstrated that alendronate, contain nitrogen which caused damage to the gastric mucosa and impaired the healing of chronic gastric ulcers (Amagase *et al.,* 2011).

Several mechanisms underlying development of osteoporosis were proposed. Wang *et al.* (2011) stated that the receptor activator of the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) ligand (RANKL), a key promoting factor for osteoclast differentiation, is expressed on osteoblastic cells. Clinical application of RANKL inhibition has a major effect on metabolic bone disease such as osteoporosis. Osteoprotegrin (OPG) inhibits RANKL-RANK pathway through competitive bindings to RANKL. OPG deficiency resulted in severe osteoporosis. Moreover, the experimental data support the idea that compounds inhibiting expression or activity of inducible nitric oxide synthase (iNOS) are potential anti-inflammatory agents (Hamalainen, *et al.,*2007)

In addition, Kim *et al.* (2006) mentioned the relationship between oxidative stress and bone mineral density or osteoporosis. They stated that the reactive oxygen species (ROS) might be relevant to osteoclast differentiation, which requires RANKL. TNF-α, frequently present in inflammatory conditions, has a profound synergy with RANKL in osteoclastogenesis. Increase in osteoclast number led to bone loss occurring in osteoporosis and inflammatory diseases. Furthermore, Braun *et al.* (2011) mentioned that an increase oxidative stress is related to osteoblasts cell death.

The development of new dietary adjuncts and novel antiosteoporotic agents which reinstate a normal metabolic environment, thereby reducing the long term complications associated with osteoporosis is required. Such as agents would ideally reduce the inflammation and ROS or those working on estrogenic receptors and increase estrogen level (Limer *et al.,* 2004 and, Ha *et al.,* 2006, Lewiecki, 2009, Smientana, *et al.,* 2010).

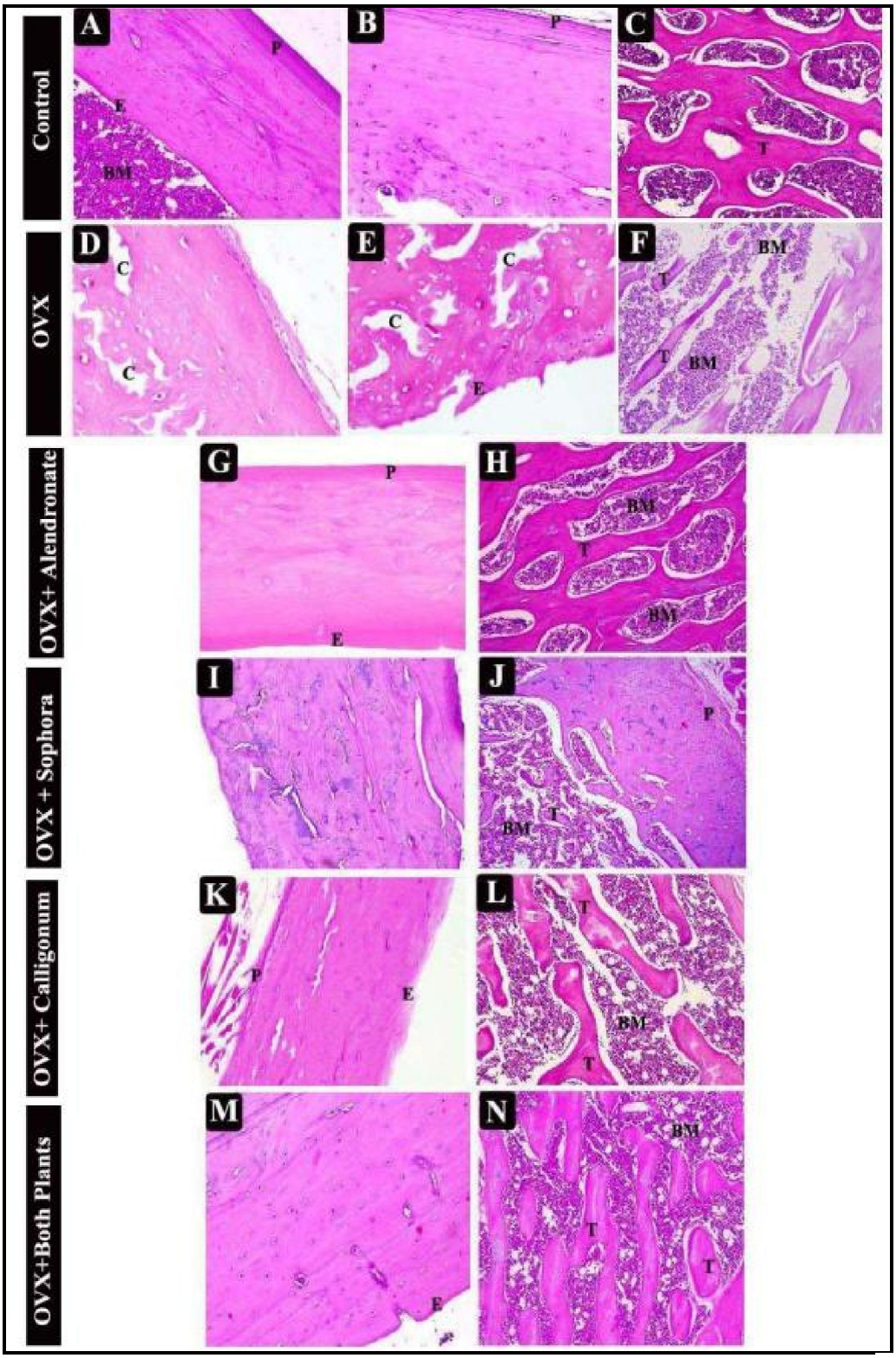

Figure 1: Photomicrography illustrating hematoxylin and eosin-stained sections of distal tibia diaphysis; Control (A/C), untreated OVX (D/F), OVX treated by alendronate (G/H), OVX treated by Sophora (I/J), OVX treated by calligonum (K/L), OVX treated by both plants (M/N). BM= bone marrow, T= trabecula, P= periosteum, E= endosteum, C= resorption cavities. Original magnification ×200 for A,B,G,I- ×100 for C,F,H,J,K,L- ×400 for D,E,M.

Sophora japonica (contains genestin, kaempferol, quercetin, and Sophoricosides), and *Calligonum comosum* (contains kaempferol and quercetin) have been found to have antioxidant and anti-inflammatory effects (kim *et al.,* Qureshi *et al.,* 2011). These two herbs have been investigated in the current research for their antiosteoporotic activity versus alendronate in ovariectomized osteoporotic albino rats.

Hamalainen *et al.* (2007) suggested that isoflavones such as genistein and the flavonols as kaempferol and quercetin, inhibit iNOS protein and NO production in a dose-dependent manner. In addition, they inhibit the activation of NF-κB, which is a significant transcription factor for iNOS. It was found that plant extracts used in the current study contain phytoestrogenic activity. Al-Anazi *et al.* (2011) reported that genistein is an isoflavones that have been shown to conserve bone in OVX- rodent models and probably have similar conservatory effects in higher mammalian species. Kaempferol is belonging to flavonoid family being chemically classified as flavonol which has estrogenic activity. In addition, Kaempferol is known to up-regulate the expression of ERS not to bind directly to it (Miyake *et al.,* 2003). Quercetin is a flavonol, also possess phytoestrogenic activity and have the capacity to bind to both ERS subtypes (Wong and Rabie, 2008).

In the present study, the body weight increase observed after ovariectomy in OVX-control group might be referred to decreased estrogen level leading to deposition of abdominal adipose tissue (Wegorzewska *et al.,* 2008). Moreover, the decreased in body weight achieved in the OVX- treatment groups compared with OVX-control group could be explained by the effect of these plants on ERS. This was in agreement with Saunier *et al.* (2011) who attributed this to the fact that plant extracts that have estrogenic activity reverse weight gain and fat accumulation. In addition, the findings of the present study that the body weight of Sophora Japonica treated group was decreased more than *Calligonum comosum* treated group could be attributed mainly to the ability of this plant to decrease in the number of large adipocytes and a concomitant increase in the number of small adipocytes (Park *et al.,* 2009).

In the current study, The non significant ($p>0.05$) decrease in serum calcium level and a non significant ($p>0.05$) increase in serum phosphorus level observed in non treated OVX-control rats could be explained by results reported by Mattix *et al.* (2003) who published that ovariectomy in rats resulted in an impaired calcium balance which could also have contributed to ovariectomy-induced osteoporosis. Moreover, the balance between calcium and phosphorous resulted from an inverse relationship. When serum calcium levels rise, phosphorous levels fall and vice versa. These two electrolytes perform similar functions, are regulated by similar body mechanism (Dhingra *et al.,* 2007).

Furthermore, the decrease in total serum calcium shown in the present work after oral treatment with alendronate and plant extracts indicated that these groups fix calcium in bone cells, and in addition, remove it from the other tissue structures. These results are in accordance to the study of Miyake *et al.* (2003) who stated that the estrogenic plant product, kaempferol, potently enhanced calcium deposition on murine pre-osteoblastic cell line MC3T3-E1, after inducing the activity of alkaline phosphatase.

In the current investigation, serum alkaline phosphatase was significantly ($p<0.05$) increased in the non treated OVX-rats, indicating an increase in bone formation which supports the results of other authors (Saleh and Saleh, 2011). Moreover, the highest level of serum alkaline phosphatase was observed in Sophora Japonica treated group than other treated groups. This might be explained by the finding of Almasan *et al*. (2011) who stated that alkaline phosphatase of bone origin is the only enzyme secreted by osteoblast with practical importance for bone pathology and increase in serum when there is a reaction associated with bone formation or repair. While a decline of serum alkaline phosphatase after three months of treatment in other treated groups mean stabilization of bone repair process.

In the present study, the increased in serum parathyroid hormone level observed in OVX treated groups could be explained as a compensatory mechanism with serum calcium level decrease. This was in agreement with the findings of Vezzoli *et al.* (2011) study which mentioned that the increase in extracellular calcium concentrations stimulates the calcium-sensing receptor and inhibits parathyroid hormone secretion and cellular proliferation.

The highly significant decrease in thickness of outer cortical bone and inner cancellous bone trabeculae in the distal tibia metaphysis and diaphysis observed in non treated OVX-control group was in agreement with other authors (Weber *et al.*, 2004 and Orlic *et al.*, 2007). Decrease of trabecular bone caused widening of the bone marrow spaces as a result of the increase in the intertrabecular distance. Similarly, Seeman (2003) mentioned that estrogen deficiency at menopause led to more resorption and less bone formation causing cortical thinning and trabecular thinning with discontinuity. Some workers from their in vitro study concluded that estrogen suppressed parathyroid hormone stimulated osteoclast like cell formation and added that estrogen

might regulate resorption by blocking attachment of osteoclast to bone (Liu, *et al.*, 2002).

Cement lines are defined as layers of matrix laid down whenever a period of resorption was followed by new bone deposition (Shaker, *et al.*, 2005). In the current study less distinct cement lines were detected following ovariectomy. This was in accordance to the findings of another study which attributed this to the fact that at menopause the rate of bone resorption exceeded that of bone formation (Seeman, 2002).

In the present study, the morphometric and histological results revealed that both plants treated groups had restored architecture of the cortical and trabecular structure with well recognized bone matrix. Moreover, they give non significant change with the control group and showed more potency comparing to each plant separately. Moreover, *Calligonum comosum* treated groups showed more potent increase of cortical and trabecular bone thickness than Sophora Japonica treated groups. In addition, a dose of 200mg/kg/day of each plant extract was more potent than a dose of 100mg/kg/day on cortical and trabecular thickness. Thus, the improvement resulted was a dose dependent. This was in accordance to Siddiqui *et al.* (2011) study which attributed quercetin to the increased bone mineral density osteoprogenitors, bone mieral density, bone formation rate, cortical deposition and improved trabecular microarchitecture. Also, Wang *et al.* (2006) study found that the low and medium dosage of genistin from *Sophora japonica* improves the femur and tibia bone densities of OVX rats in 12 weeks treatment.

Combination of both plants was found to cause significant improvement in biochemical markers, moprphometric, and histological examination compared to non treated OVX-control group. Moreover, it produced a non-significant change compared to normal control group. This result was very near with the result of alendronate treated group.

It might be suggested that the beneficial synergistic effect of the combination of both plants is due to additive antioxidant, anti-inflammatory, and phytoestrogenic actions.

In conclusion, despite the limitation of the study, it can be concluded that the administration of the plant extracts of *Sophora japonica* and *Calligonum comosum*, whether alone or in combination, may be beneficial when used in osteoporosis and thus might be promising therapeutic agents.

References

1. Al-Anazi, A.F., Qureshi, V.F., Javaid K, Qureshi, S., (2011): Preventive effects of phytoestrogens against postmenopausal osteoporosis as compared to the available therapeutic choices: An overview. J Nat Sci Biol Med., 2(2): 154–163.
2. Almasan, H., Băciuţ, G., Băciuţ, M., Almăşan, O., Bran, S., Oana, L., (2011):Serum changes induced by intramedullar experimental administration of bisphosphonates. Rom J Morphol Embryol., 52:435–442.
3. Amagase, K., Inaba, A., Senta, T., Ishikawa, Y., Nukui, K., Murakami, T., Takeuchi, K., (2011): Gastric ulcerogenic and healing impairment effects of risedronate, a nitrogen-containing bisphosphonate in rats. Comparison with alendronate and minodronate. J Physiol Pharmacol., 62(6):609-18.
4. Badria, F.A., Ameen, M., Akl, M.R., (2007): Evaluation of cytotoxic compounds from *Calligonum comosum* L. growing in Egypt. Z Naturfrosch C., 62(10):656-60.
5. Bhathena SJ and Velasquez MT (2002); Beneficial role of dietary phytoestrogens in obesity and diabetes. Am J Clin Nutr. 76(6):1191-201.
6. Braun, K.F., Ehnert, S., Freude, T., Egaña, J.T., Schenck, T.L., Buchholz, A., Schmitt, A., Siebenlist, S., Schyschka, L., Neumaier, M., Stöckle, U., Nussler, A.K., (2011): Quercetin protects primary human osteoblasts exposed to cigarette smoke through activation of the antioxidative enzymes HO-1 and SOD-1. Scientific World Journal., 11:2348-57.
7. Chen, H.N**.,** and Hsieh, C.L., (2010): Effects of *Sophora japonica* flowers (Huaihua) on cerebral infarction,Chin Med**.,** 27:5-34.
8. Dhingra, R., Sullivan, L.M., Fox, C.S., Wang, T.J., Gaziano, J.M., Vasan, R.S., (2007): Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. Arch Intern Med.,14;167(9):879-85.
9. Graham, L.S., Parahami, F., Tintut, Y., Kitchen, C.M., Demer, L.L., and Effors, R.B. (2009): Oxidized lipids enhance RANKL production by T lymphocytes: implications for lipid-induced bone loss. Clin Immunol. 133(2):265-75.
10. El-Hawary ZM and Kholief TS (1990): Biochemical studies on some hypoglycemic agents (II) effect of *Calligonum comosum* extract. Arch. Pharm. Res. (13): 113-116.
11. Ha, H., Lee, Y.S., Lee, J.H., Choi, H., Kim, C., (2006): High performance liquid chromatographic analysis of isoflavones in

medicinal herbs. Arch Pharm Res., 29(1):96-101.
12. Hamalainen, M., Nieminen, R., Vuorela, P., Heinonen, M., Moilanen, E., (2007): Anti-Inflammatory Effects of Flavonoids: Genistein, Kaempferol, Quercetin, and Daidzein Inhibit STAT-1 and NF-κB Activations, Whereas Flavone, Isorhamnetin, Naringenin, and Pelargonidin Inhibit only NF-κB Activation along with Their Inhibitory Effect on iNOS Expression and NO Production in Activated Macrophages. Mediators Inflamm., 45673:1-10.
13. Jakob F, Seefried L, Ebert R. (2008): [Pathophysiology of bone metabolism]. **Internist (Berl).**
14. Oct;49(10):1159-60, 1162, 1164 passim. doi: 10.1007/s00108-008-2113-0.
15. Kapur P., Harry H., Wuttke W. *et al.* (2008): evaluation of the antiosteoporotic potential of Tinospora Cordifolia in female rats. Maturitas 20;59(4):329-38
16. Kim BH, Chung EY, Min BK, Lee SH, Kim MK, Min KR, Kim Y (2003); Anti-inflamatory action of legue isoflavonoid sophoricoside through inhibition on cyclooxygenase-2 activity. Planta Med. 69(5):474-6.
17. Kim, H.J., Chang, E.J., Kim, H.M., Lee, S.B., Kim, H.D., SuKim, G., Kim, H.H., (2006): Antioxidant alpha-lipoic acid inhibits osteoclast differentiation by reducing nuclear factorkappa BDNA binding and prevents *in vivo* bone resorption induced by receptor activator of nuclear factor-kappaB ligand and tumor necrosis factoralpha. Free Radic Biol Med., 1;40(9):1483-93.
18. Kim, J.M., Lee, E.K., Kim, D.H., Yu, B.P., Chung, H.Y., (2010): Kaempferol modulates pro-inflammatory NF-kappaB activation by suppressing advanced glycation endproducts-induced NADPH oxidase. Age (Dordr)., 32(2):197-208.
19. Lacativa PG and Farias ML (2010); Osteoporosis and inflammation. Arq Bras Endocrinol Metabol. 54(2):123-32.
20. Lewiecki, E.M., (2009): Lasofoxifene for the prevention and treatment of postmenopausal osteoporosis. Ther Clin Risk Manag., (5):817-27.
21. Limer, J.L., and Speirs, V., (2004): Phyto-oestrogens and breast cancer chemoprevention. Breast Cancer Res., 6 (3):119–27.
22. Liu XM, Zakaria MN, Islam MW, Radhakrishnan R, Ismail A, Chen HB, Chan K, AL-Attas A (2001); Anti-inflammatory and anti-ulcer activity of *Calligonum comosum* in rats. Fitoterapia. 72(5):487-91.
23. Liu, B.Y., Wu, P.W., Bringhurst, F.R, and Wang, J.T., (2002): Estrogen inhibition of PTH stimulated osteoclast formation and attachment. *in vitro*: involvement of both PKA and PKC. Endocrinology.143 (2): 627-35
24. Mattix Kramer, H.J., Grodstein, F., Stampfer, M.J., Curhan, G.C., (2003): Menopause and postmenopausal hormone use and risk of incident kidney stones. J Am Soc Nephrol., (14):1272-1277.
25. Miyake, M., Arai, N., Ushio, S., Iwaki, K., Ikeda, M., Kurimoto, M., (2003): Promoting effect of kaempferol on the differentiation and mineralization of murine pre-osteoblastic cell line MC3T3-E1. Biosci Biotechnol Biochem., 67 (6):1199-205.
26. Neumann E. (2006): New pathophysological relevant metabolic pathways in osteoporosis: Future innovative therapies. Z Rheumatol. Sep**;** 65(5):400, 402-6.
27. Orlic, I., Borovecki, F., Simic, P., Vukicevic, S., (2007): Gene expression profiling in bone tissue of osteoporotic mice. Arh Hig Rada Toksikol. 58:3–11.
28. Paget, L.H., and Barnes, O., (1964): Evaluation of Drug Activities: Pharmacometrics by Laurence and Bacharach, Academic Press, NY, USA. Vol. I, PP. 90-91
29. Park. K.W., Lee, J.E., Park, K.M., (2009): Diets containing Sophora japonica L. prevent weight gain in high-fat diet-induced obese mice. Nutr Res., 29(11):819-24.
30. Qureshi, A.A**.**, Tan, X**.**, Reis, J.C**.**, Badr, M.Z**.**, Papasian, C.J**.**, Morrison, D.C**.**, Qureshi, N**.,** (2011): Inhibition of nitric oxide in LPS-stimulated macrophages of young and senescent mice by δ-tocotrienol and quercetin. Lipids Health Dis**.,** (20)10-239.
31. Rizzoli, R**.,** (2011): Bisphosphonates for post-menopausal osteoporosis: are they all the same?. QJM., 104(4):281-300.
32. Saleh. N.K**.**, Saleh. H.A**.,** (2011): Olive oil effectively mitigates ovariectomy-induced osteoporosis in rats. BMC Complement Altern Med**.,** (4):11:10.
33. Saunier, E.F., Vivar, O.I., Rubenstein, A., Zhao, X., Olshansky, M., Baggett, S., Staub, R.E., Tagliaferri, M., Cohen, I., Speed, T.P., Baxter, J.D., Leitman, D.C., (2011): Estrogenic plant extracts reverse weight gain and fat accumulation without causing mammary gland or uterine proliferation., 6(12): 1-9.
34. Seeman, E., (2002): Pathogenesis of bone fragility in women and men. Lancet.,(50): 359: 1841.

35. Seeman, E.O., (2003): Invited Review: Pathogenesis of osteoporosis. J. Appl. Physioi., 95(5): 2142-2151.
36. Shaker, S.A., Mohamed, G.F., Saleh, H.A., and Seif, A., (2005): histological study on the effect of calcium supplementation in prophylaxis and treatment of osteoporosis in ovariectomized rats. The Egyptian journal of histology., 2(28): 191-198.
37. Siddiqui, J.A., Swarnkar, G., Sharan, K., Chakravarti, B., Gautam, A.K., Rawat, P., Kumar, M., Gupta, V., Manickavasagam, L., Dwived,i A.K., Maurya, R., Chattopadhyay, N., (2011): A naturally occurring rare analog of quercetin promotes peak bone mass achievement and exerts anabolic effect on osteoporotic bone. Osteoporos Int., 22(12):3013-27.
38. Smietana, M.J., Arruda, E.M., Faulkner, J.A., Brooks, S.V., Larkin, L.M. (2010): Reactive oxygen species on bone mineral density and mechanics in Cu,Zn superoxide dismutase (Sod1) knockout mice. Biochem Biophys Res Commun., 3;403(1):149-53.
39. Tang, Y.P., Li, Y.F., Hu, J., Lou, F.C., (2002): Isolation and identification of antioxidants from Sophora Japonica. J Asian Nat Prod Res., 4(2):123-8.
40. Vezzoli, G., Terranegra, A., Rainone, F., Arcidiacono, T., Cozzolino, M., Aloia, A., Dogliotti, E., Cusi, D., Soldati, L., (2011): Calcium-sensing receptor and calcium kidney stones., J Transl Med.,(22)9:201.
41. Wang, ZL., Sun, J.Y., Wang, D.N., Xie, Y.H., Wang, S.W., Zhao, W.M., (2006): Pharmacological studies of the large-scaled purified genistein from Huaijiao (*Sophora japonica*-Leguminosae) on anti-osteoporosis. Phytomedicine., 13(10):718-23.
42. Wang, Y., Huang, P., Tang, P.F., Chan, K.M., Li, G., (2011): Alendronate (ALN) combined with osteoprotegerin (OPG) significantly improves mechanical properties of long bone than the single use of ALN or OPG in the ovariectomized rats. J Orthop Surg Res., (13) 6-34.
43. Weber, K., Kaschig, C., Erben, R.G., (2004): 1a-Hydroxyvitamin D2 and 1a-hydroxyvitamin D 3 have anabolic effects on cortical bone, but induce intracortical remodeling at toxic doses in ovariectomized rats. Bone., 35(3):704–710.
44. Wegorzewska, I.N., Walters, K., Weiser, M.J., Cruthirds, D.F., Ewell, E., Larco, D.O., Handa, R.J., Wu, T.J., (2008): Postovariectomy weight gain in female rats is reversed by estrogen receptor alpha agonist, propylpyrazoletriol. Am J Obstet Gynecol.,199(1):67 -5.
45. Wong, R.W., Rabie, A.B., (2008): Effect of quercetin on preosteoblasts and bone defects, Open Orthop J; 10(2):27-32.

10/5/2013